



Evaluation of the micro-gravity compatible pre-concentrator system for the microbial monitoring of International Space Station water samples

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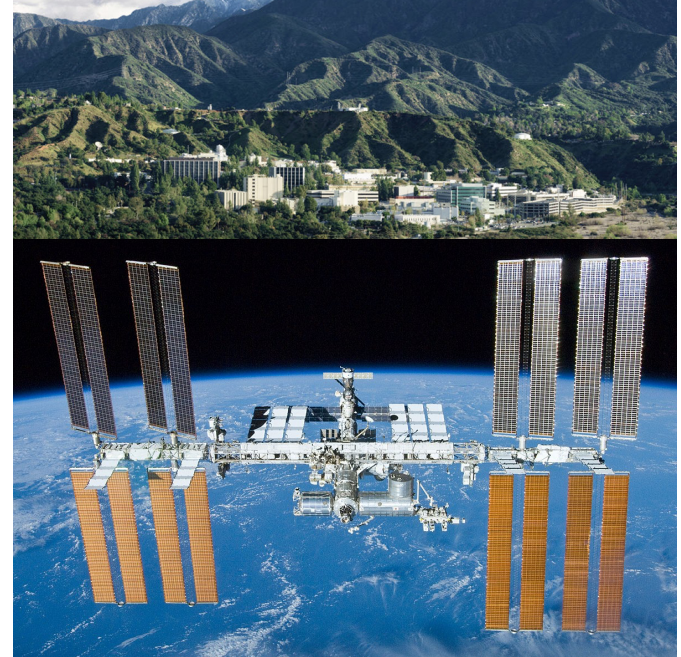
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Overview

- Background
- Concentrator devices
- Methods for testing efficiency
- CFU and qPCR Data
- Other work done at JPL



Problems faced with Microbial Monitoring on the ISS

- Current microbial monitoring methods for ISS water samples are laborious and time-consuming.
- Technological advancement of rapid microbial detection and identification systems have far outpaced sample preparation techniques.
- Sample concentration technologies are needed to meet NASA Medical Operation Requirement Document (MORD) specification for potable water
 - 5×10^4 CFU/liter.



Current Concentrator Devices

- Millipore
 - Membrane Filter
 - Plate or suspend in desired fluid
- Innovaprep CP-150
 - Hollow fiber membrane filter
 - No transfer steps needed
 - High concentration factor
 - Wet-foam elution



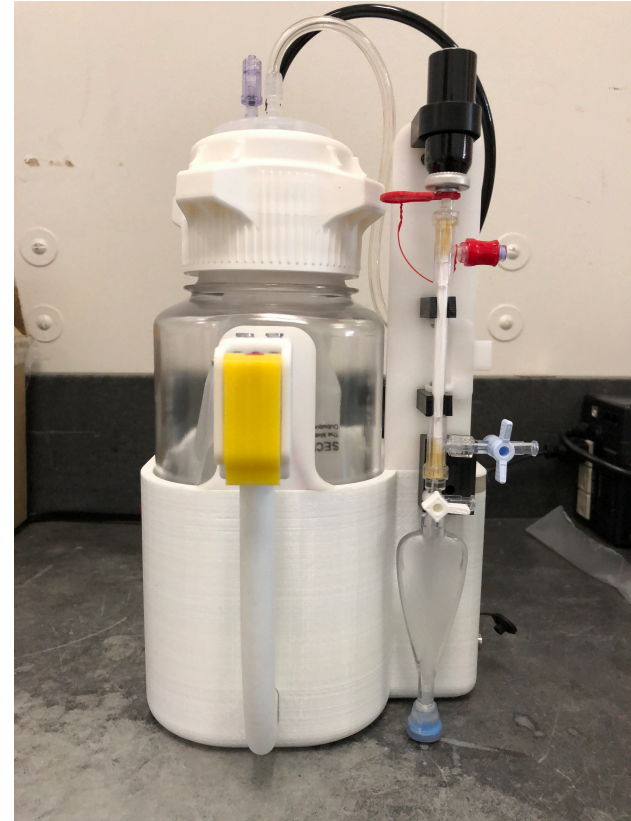
Development of the iSSC

- iSSC stands for the International Space Station Smart Sample Concentrator.
- Provide ISS capability for rapid concentration of microorganisms.
- Concentrate 1L samples into as low as 250 μL using hollow fiber membrane filtration and wet-foam elution.

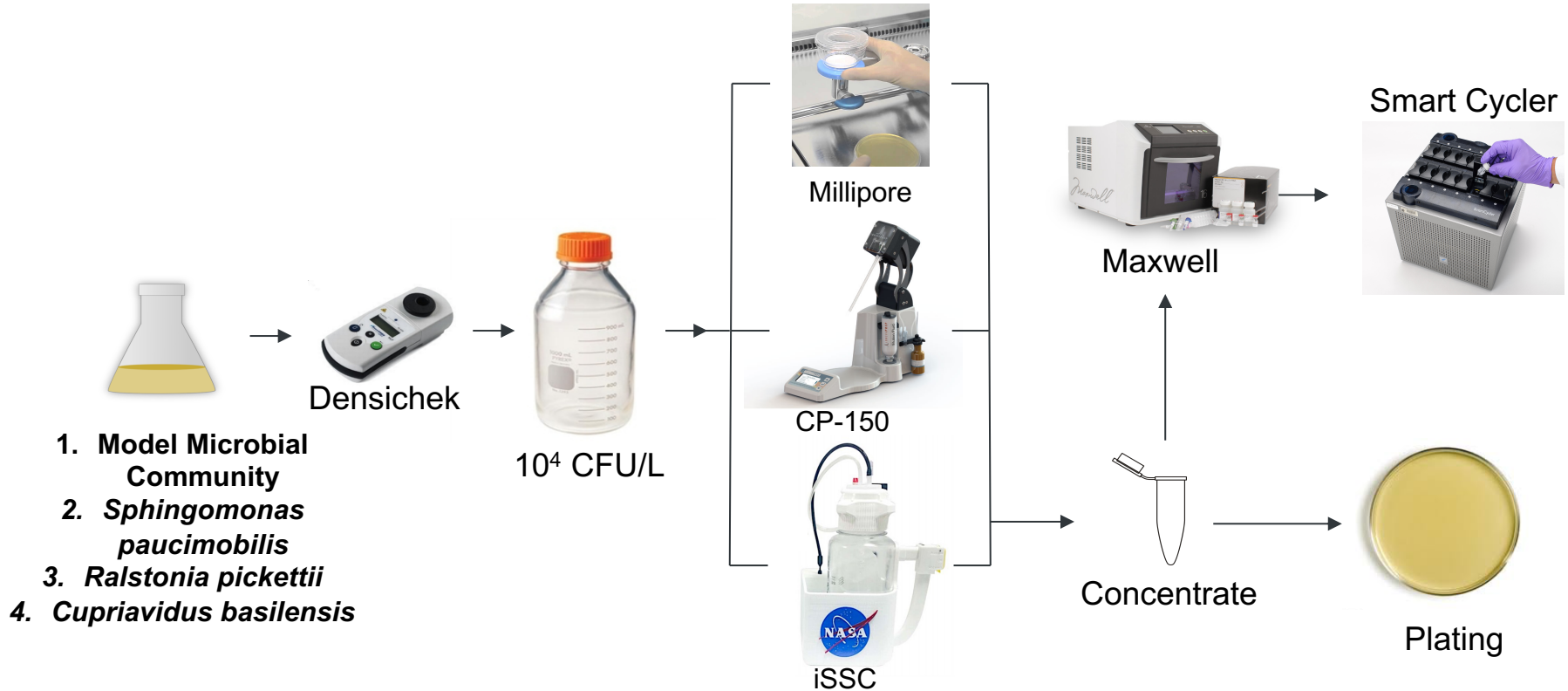


How did we test the efficiency of the iSSC?

- iSSC was compared against Innovaprep CP-150 and Millipore 0.45 μ m filters.
- 1 bacterial community and 3 model organisms were used to test the efficiency of each machine.
- Data obtained includes CFU counts, qPCR data of the 16S rRNA gene.

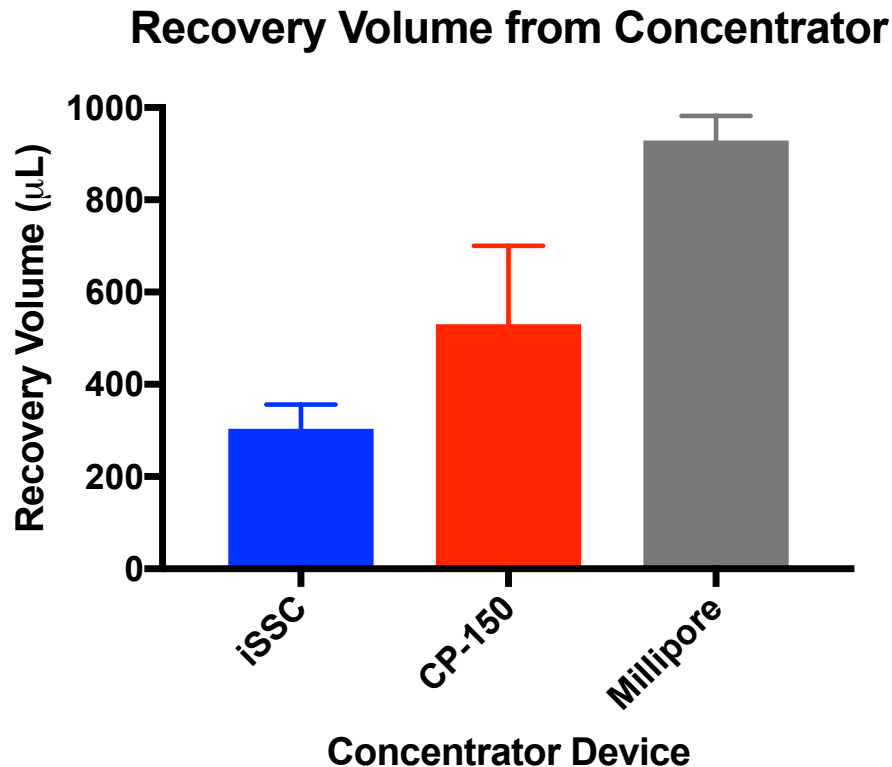


Methods Employed to Determine Concentrator Efficiency



Efficacy of iSSC system in the detection of microbial cells at the concentration of 10^4 cells per liter of water

Average Concentration for iSSC, CP-150, and Millipore

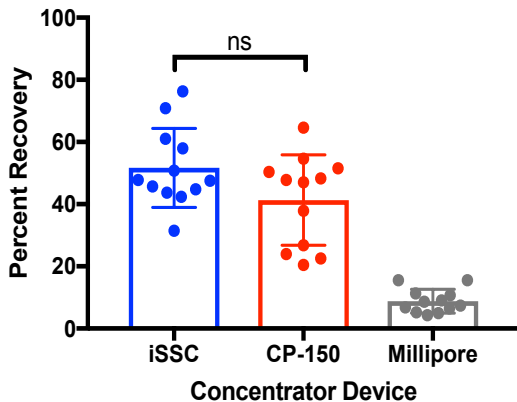


The average recovery volume after concentration using iSSC was 304 μL (n=9), CP-150 was 530 μL (n=9), and Millipore was 928 μL (n=9).

Percent Recovery based on colony count using 10^4 CFU/ml data

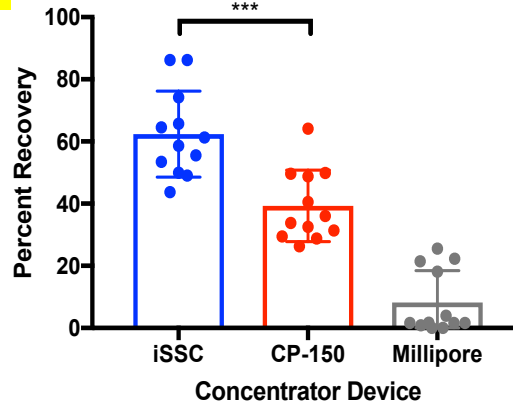
CFU

Percent Recovery of *S. paucimobilis*



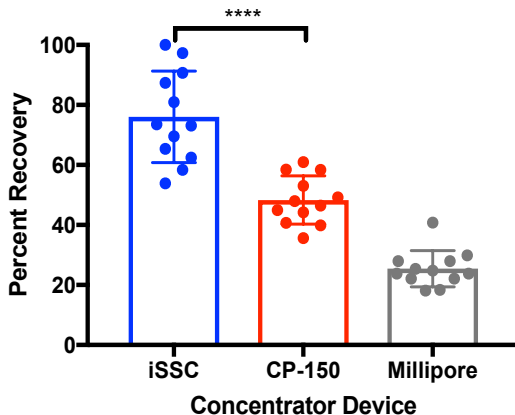
The average percent recovery of *S. paucimobilis* for iSSC was 52%, CP-150 was 41%, and Millipore was 9%, $p=0.0769$, $n=12$.

Percent Recovery of *R. pickettii*



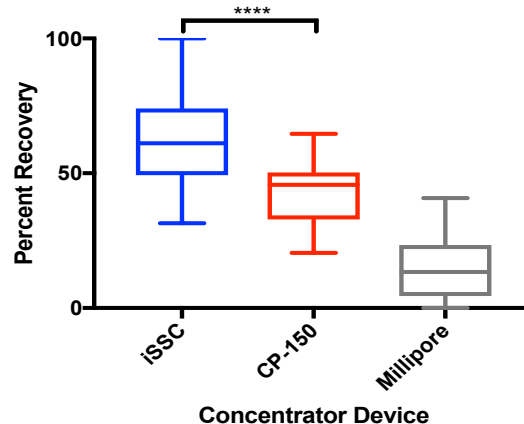
The average percent recovery of *R. pickettii* for iSSC was 62%, CP-150 was 39%, and Millipore was 8%, $p=0.0002$, $n=12$.

Percent Recovery of *C. basilensis*



The average percent recovery of *C. basilensis* for iSSC was 76%, CP-150 was 48%, and Millipore was 25%, $p<0.0001$, $n=12$.

% Recovery of 3 Bacterial Populations

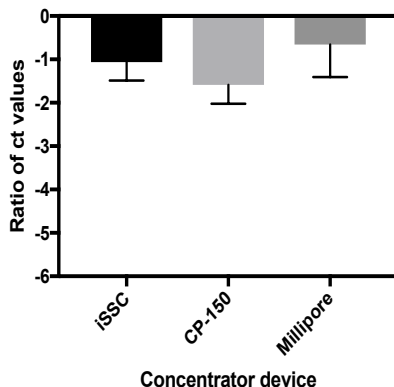


The average percent recovery of all bacterial communities for iSSC was 63%, CP-150 was 43%, and Millipore was 14%, $p<0.0001$, $n=36$.

Concentration efficiency using Smart cyclers qPCR data

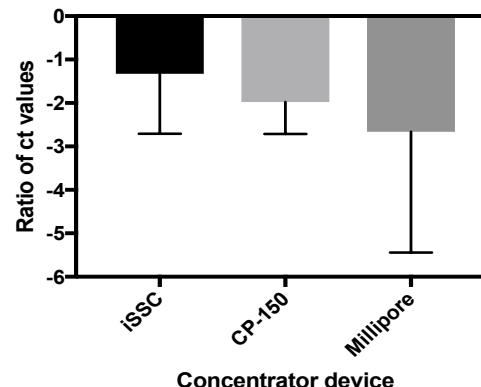
qPCR

Sphingomonas paucimobilis Smart cyclers ct value data



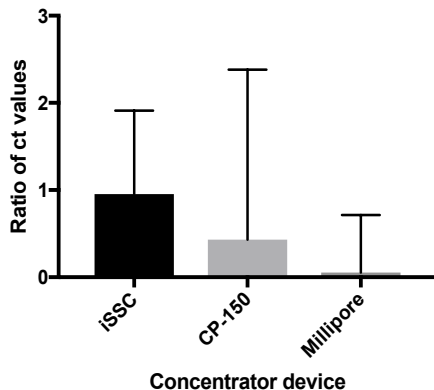
S. paucimobilis:
Average difference in Ct values for iSSC was 1.5, CP-150 was 2.5, and Millipore was 1.
1 log difference in copy # will be ~3 Ct
n=12.

Ralstonia pickettii Smart cyclers ct value data



R. pickettii:
Average difference in Ct values for iSSC was 1.5, CP-150 was 2.0, and Millipore was 3.
1 log difference in copy # will be ~3 Ct
n=12.

Cupriavidus basilensis Smart cyclers ct value data



C. basilensis:
Average difference in Ct values for iSSC was 1.5, CP-150 was 2.5, and Millipore was 1.
1 log difference in copy # will be ~3 Ct
n=12.

Since the copy number variations are enormous, DNA extraction efficiency, higher concentration of target DNA, and other PCR platforms (RAZOR, Light Cycler, BioRad) are explored

Model Microbial Community Composition

TABLE 1. Various characteristics of MMC constituents

Sample no.	Microbe	Strain	Domain: phylum	Morphology	Culture conditions or reference ^a	Incubation time (h)	Other available source(s) ^b	Significance (reference[s])
1	<i>Aureobasidium pullulans</i>	28v1	<i>Eukarya: Ascomycota</i>	Black yeast	TYG agar, 30°C	86	NRRL 58992	Eukaryotic representative, facultative anaerobic spore former, original isolate (16)
2	<i>Acinetobacter radioresistens</i>	50v1	<i>Bacteria: Gammaproteobacteria</i>	Short rods	TYG agar, 32°C	60	NRRL B-59417	Resistant to desiccation and radiation, aerobic non-spore former, original isolate (16)
3	<i>Bacillus megaterium</i>	KL-197	<i>Bacteria: Firmicutes</i>	Rods	TYG agar, 32°C	38	NRRL B-59415	Frequently isolated from SAC (15, 16), facultative anaerobic spore former, vegetative cells of original isolate
4	<i>Bacillus pumilus</i>	SAFR-032	<i>Bacteria: Firmicutes</i>	Spores	See reference 13	NA ^c	ATCC PTA-7603, NRRL B-30938	Strains showing extraordinary UV resistance, frequently isolated from SAC (13), facultative anaerobic spore former, spores of original isolate
5	<i>Deinococcus radiodurans</i>	ATCC 13939	<i>Bacteria: Deinococcus-Thermus</i>	Cocci, tetrad	TYG agar, 32°C	60	NRRL B-59418, DSM 20539	Strains showing extraordinary gamma radiation resistance, sequences retrieved from spacecraft surfaces (3), aerobic non-spore former, isolate procured from culture collection
6	<i>Microbacterium imperiale</i>	47v1	<i>Bacteria: Actinobacteria</i>	Short rods	TYG agar, 32°C	60	NRRL B-59416	Extremely hardy, difficult to extract DNA from cells (17), aerobic non-spore former, original isolate
7	<i>Staphylococcus wamneri</i>	82-4	<i>Bacteria: Firmicutes</i>	Cocci	TYG agar, 32°C	40	NRRL B-59414	Frequently isolated from SAC (16, 35), human associated, facultative anaerobic non-spore former, original isolate
8	<i>Micrococcus luteus</i>	ATCC 4698	<i>Bacteria: Actinobacteria</i>	Rods	Nutrient agar, 32°C	60	NRRL B-59413, DSM 20030	Common contaminant of indoor environments and SAC (16), air borne and human associated, aerobic non-spore former, original isolate
9	<i>Cupriavidus metallidurans</i>	CH34 ^d	<i>Bacteria: Betaproteobacteria</i>	Rods	TYG agar, 32°C	60	ATCC 43123	Ubiquitously isolated from SAC (22), heavy metal resistant, aerobic non-spore former
10	<i>Clostridium sporosphaeroides</i>	DSM 1294	<i>Bacteria: Firmicutes</i>	Anaerobic rods	DSMZ medium 78, 37°C, anaerobic	48	ATCC 25781	Representative of obligate anaerobic spore formers found in SAC (30, 35), isolate procured from culture collection, vegetative cells
11	<i>Methanobacterium formicicum</i>	DSM 1535	<i>Archaea: Euryarchaeota</i>	Rods	DSMZ medium 119, 37°C, anaerobic	72	ATCC 33274	Representative of broad (uncultivated) archaeal diversity of SAC (21, 23), anaerobic non-spore former, isolate procured from culture collection

^a TYG, tryptone yeast extract glucose.

^b ATCC, American Type Culture Collection; NRRL, USDA culture collection; DSM, German collection of cell cultures; PTA, patented strain.

^c NA, not applicable since the spores were prepared prior to the investigation and the appropriate concentration from the original stock was used.

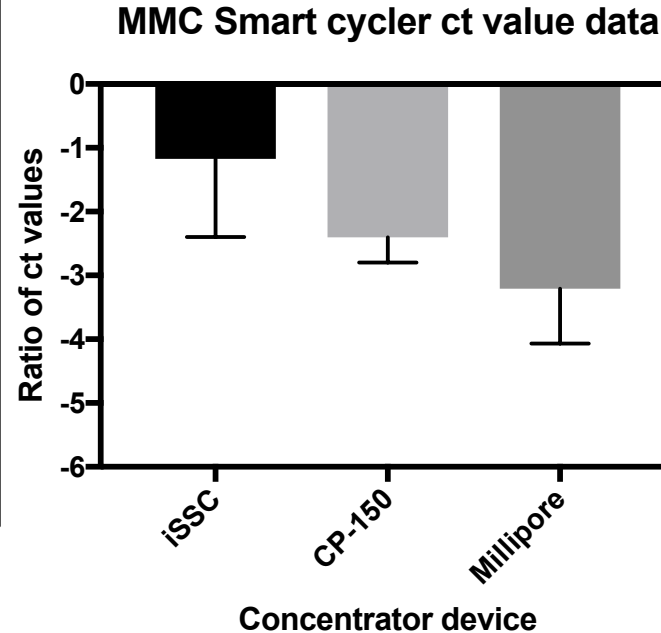
^d Received from Natalie Leys, Hoofd Onderzoeks Eenheid Microbiologie at SCK-CEN, Antwerp, Belgium.

Concentration efficiency of Model Microbial Community using qPCR data

MMC:

Average difference in Ct values for iSSC was 1.25, CP-150 was 2.5, and Millipore was 3.5.

1 log difference in copy # will be ~3 Ct
n=12.



Overall Conclusions and Future Directions for iSSC Project

- CFU and qPCR results suggest that the iSSC concentrated more microorganisms and relevant 16S rRNA gene copies and hence considered better sample concentrator compared to CP-150 and Millipore systems.
- Similar experiments will be repeated to test the efficiency of iSSC concentrator for 10^3 & 10^2 dilutions per liter.
- Coordinate with KSC in evaluating iSSC system for “microbial monitoring” applications using RAZOR.

Acknowledgements

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